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for

NASA's Platform for Cross-Disciplinary Microchannel Research

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NASA's Platform for Cross-Disciplinary Microchannel Research

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Abstract

A team from the Structural Biology group located at the NASA Marshall Space Flight Center in Huntsville, Alabama is developing a platform suitable for cross-disciplinary microchannel research. The original objective of this engineering development effort was to deliver a multi-user flight-certified facility for iterative investigations of protein crystal growth; that is, Iterative Biological Crystallization (IBC). However, the unique capabilities of this facility are not limited to the low-gravity structural biology research community. Microchannel-based research in a number of other areas may be greatly accelerated through use of this facility. In particular, the potential for gas-liquid flow investigations and cellular biological research utilizing the exceptional pressure control and simplified coupling to macroscale diagnostics inherent in the IBC facility will be discussed. In conclusion, the opportunities for research-specific modifications to the microchannel configuration, control, and diagnostics will be discussed.

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A PLATFORM FOR CROSS-DISCIPLINARY MICROCHANNEL RESEARCH

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ABSTRACT

A team from the structural biology group located at the Marshall Space Flight Center in Huntsville Alabama is developing a platform suitable for cross-disciplinary microchannel research. The original objective of this engineering development effort was to deliver a multi-user flight-certified facility for iterative investigations of protein crystal growth; that is, Iterative Biological Crystallization. However, the unique capabilities of this facility are not limited to the low-gravity structural biology research community. Microchannel-based research in a number of other areas may be greatly accelerated through use of this facility. In particular, the potential for gas-liquid flow investigations and cellular biological research utilizing the exceptional pressure control and simplified coupling to macroscale diagnostics inherent with the facility will be discussed. Mention of the opportunities for research-specific modifications to the microchannel configuration, control and diagnostics will also be made.

INTRODUCTION

For nearly two decades, structural biologists have attempted to utilize the low-gravity conditions in space to grow both larger, and more ordered crystals of proteins, viruses, and nucleic acids for x-ray diffraction study. The diffraction pattern is needed to determine the 3-dimensional positioning of all the atoms within a molecule, thus allowing the elucidation of the structure. Once this is known, the structure/function relationship can aid others in uncovering the mechanism for disease and possibly lead to new drug design (structure based drug design). Despite the fact that the hardware used for such studies in space has been non-optimized and limited in sample numbers, the program has seen many successes, Kundrot et.al. (2001). In fact, looking at just those systems flown on the Space Shuttle, about 20% of them resulted in the investigator obtaining the best diffraction data set ever, as compared to any other method used thus far. Also noteworthy was that with the opportunity to re-fly experiments, the investigators chances at success increased dramatically Kundrot et. al. (2001). The improved crystal quality has been attributed to both reduced buoyancy driven convection or elimination of sedimentation effects. However, experiments that are more conclusive are needed to definitively show a relationship between the acceleration levels and overall crystal quality.

The efforts reported on here, were initiated to introduce new hardware into the space program that would allow crystal growth to be done similar to how it is done in a ground-based laboratory. The goals were to increase the number of samples available, reduce the volumes of materials needed, enable automation, and allow for visual inspection. All this, plus be able to mix up the next set of complex mixtures on orbit and begin again (something that had not been an option in the past), hence the name Iterative Biological Crystallization (IBC). Very quickly lab-on-a-chip technology was chosen as the optimal method for mixing and dispensing the small volumes of costly biological materials that comprise the solutions necessary for growth. Most attractive, was the reduction in volume, mass and power offered by the technique. However, in short order the reproducibility, accuracy, and amenability to automation followed as added bonuses.

IBC Lab-On-a-Chip Design

Since lab-on-a-chip devices were not available for such studies, IBC undertook to compile the requirements needed to develop one. A collaboration referred to as the Application Developers Program (ADP) was established with Caliper Technologies Corp. (Mountain View, CA) in order to design and manufacture a chip device specifically for our application. As a participant in the ADP, IBC was provided with application development hardware, and chip design, fabrication, and manufacturing support. Considerations for the initial chip design included the viscosities of fluids, the ratios of mixtures, the order of solution addition, and the volume among others. Taking into consideration our science requirements, and the constraints of the microfluidic workstation used, the Caliper 42, we designed a chip called the NS374 shown in Figure 1. The novel chip has eight wells that each interface with the Caliper 42 instrument for independent control of pressure, voltage, or current. For our purposes, we chose to use pressure as a means of controlling the flow. We chose to design the chip to perform "batch-type" crystallizations. In this manner, constituent solutions are dispensed and mixed to exceed the supersaturation for the macromolecule.

The channels in the chip were made via standard photolithography methods and have a trough-shape as shown in Figure 2. The NS374 channel diameters range between 40-120 microns. The volume of the wells is about 27 μL. For this first chip, the design was optimized for solution viscosities of about < 5 cp, to simplify the numerous design challenges.

Given the 8 port constraint imposed by the Caliper 42, we chose to utilize 5 wells as reservoirs for reagent solutions, 2 for growth wells and 1 for waste. Constituent solutions for crystal growth were loaded onto the chip and a prescribed software script for the control of pressure at each port was utilized to dispense specified volumes of fluid from each well. The first five wells were loaded with protein stock solution, buffers, salt, and a precipitating agent. The last three wells were used for dispensing the mixtures to either one of the two growth wells, or a waste well. The chip was designed such that prior to delivery of the reaction mixture to the growth wells, complete mixing, via diffusion would have occurred. Different recipes may be delivered to two growth wells on the same chip, with a rinse to the waste well with buffer solution in between.

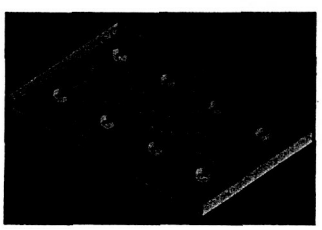


Fig. 1 Schematic of a NS374 chip developed for crystallization. Microchannels are highlighted in blue.

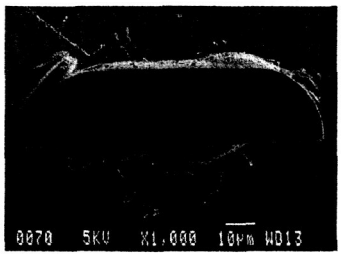


Fig. 2 Scanning electron micrograph of a typical channel cross-section. (Courtesy of Caliper Technologies Corp.).

Crystallization Results

Crystallizing the two model systems, thaumatin and glucose isomerase on the NS374, proved the efficacy of the chip and validated our approach. Figure 3 shows a glucose isomerase crystal grown in an NS374 well after dispensing solutions to be mixed via diffusion along the channels. Note the channel leading into the growth well (diameter 2.0 mm). In this particular run, the volume of solution dispensed was 10 μL and the recipe had been verified by previously testing crystallization off the chip with standard methodology (micro-batch plates).

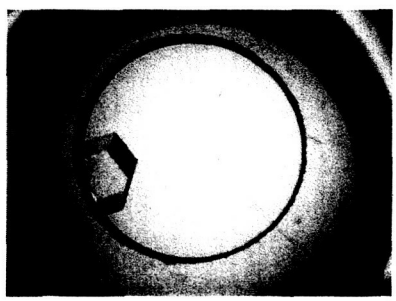


Fig. 3 Photomicrograph of a glucose isomerase crystal that has nucleated and grown in a growth well of the NS374 chip.

Current Hardware Capabilities

The unit used for multiport pressure control, the Caliper 42, has tremendous features such as accuracy, ease of use, and readily accessible ports for diagnostics. Some specifications for the unit used are as follows:

Voltage control: 100 V to 3000 V (± 1%)
Current control: -40 μA to +40 μA (± 40 nA)
Pressure Control: -5 psi to +5 psi (± 0.2%)

Both the voltage and pressure sampling rates are variable. All data can be displayed in real time and captured as well. For optical monitoring, the workstation has integrated a Nikon TE 300 inverted microscope that can house various

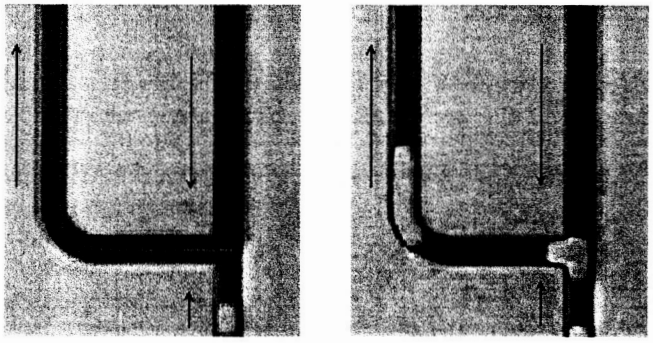
objectives as needed. We typically use either a 2X or a 10X objective. Three optical channels exist for data collection. Pressure control for the Caliper 42 can be operated manually (i.e., entered real time by the operator), or by programming a script that controls the pressure in relation to given dispensing and mixing instructions. The scripts can be written to give an almost unlimited number of possible dispense and mix scenarios. As for processing rates, for 1 cp fluid, a 2 μL droplet can flow through the NS374 chip with maximum pressure differential as quickly as a couple of minutes, or can take over an hour with the right pressure differential setting.

UTILITY FOR OTHER RESEARCH COMMUNITIES

Potential for Multi-Phase Flow Research

A number of the IBC features which are desirable for iterative protein crystal growth are also desirable for studies of multiphase flow in microchannels; such as the precise pressure control and measurement, the ability to prepare control scripts for test runs, and the ease with which the microchannels are integrated with the macro-scale diagnostics. The micro-to-macro integration eliminates many hours of time typically involved with ensuring good leak-free sealing, bubble purging, alignment of the test section with optical diagnostics, etc. In addition, to test the microfluidic response to a slight change in geometry requires only a quick change out of the chip (see Fig. 4).

Fig. 4 Photograph of the chip to microfluidic workstation interface. (Courtesy of Caliper Technologies Corp.).



(a) (b)

Fig. 5 Images of multiphase flow in channels with a cross-section as shown in Figure 2. Image (a) illustrates laminar flow of two colored fluids merging in the tee. Image (b) illustrates the disruption of this laminar flow due to gas ingestion at the tee.

Figure 5 illustrates the utility of the IBC setup for multiphase flow studies with two liquids and a gas. The image in Figure 5(a) is of laminar flow from two legs into the third leg. The ratio of fluid A (green) to that of fluid B (red) in the tee is easily controlled. The image in Figure 5 (b) is an example of how the presence and/or motion of a bubble at the junction can disrupt the fluid distribution as well as the laminar flow. The parameter range over which this type of phenomena may be investigated is easily varied and may include the use of immiscible liquids instead of a gas.

The ease of test repetition and pressure control is of great advantage when developing empirical correlations for pressure drops or flow regime mapping, Stanley (1997). Correlating the pressure drop of given flow regime through a particular microchannel geometry may require dozens of tests in order to obtain statistically significant data. Since the experiment control and acquisition is scripted, set up for repetitive experiments is only a matter of minutes. Additionally, repetitive tests may be conducted simultaneously within the same chip by taking advantage of the multiple, independently controlled ports. Parallel experiments may be conducted where either the pressure drop is varied for identical channels having varying geometry or identical pressure drops are imposed on channels having varying geometry. Either scenario, or a combination of both, is easily accomplished.

An important aspect of multiphase flow studies is the necessity for experimental reproducibility. Visualization of the complex phenomena has little engineering value if the observed phenomena cannot be predictably reproduced. An example of such complex behavior is shown in Figure 6 that illustrates an example of slug flow through the same tee section as illustrated in Figure 5. Downstream of this tee is a four-channel junction (lower left of image) in which a bubble has been trapped and the subsequent restriction has resulted in a flow regime transition. The conditions that have resulted in such complex flow phenomena are accurately reproducible using the IBC setup.

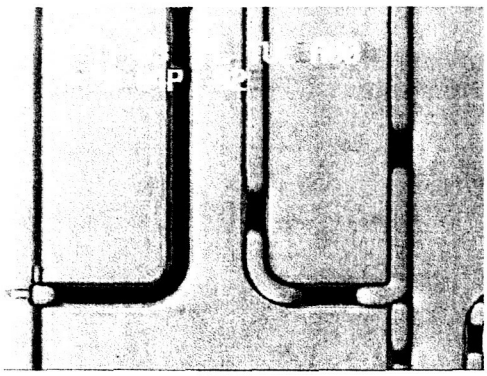


Fig. 6 Image of slug flow through a tee and the subsequent flow transition at a restriction.

To summarize, there is significant advantages in the IBC approach relative to more traditional techniques for investigating two-phase flow in microchannels. Among these advantages are the micro-to-macro interface, the flow control and the ability to conduct simultaneous, independent experiments within the same chip. These three aspects of the IBC platform allow for easy variation of test parameters (pressure, viscosity, surface tension, void fraction and geometry) and rapid acquisition of repetitive test runs.

Potential for Biological Research

Microchannel networks have been shown to be a significant improvement over more traditional analytic techniques for certain bio-analysis applications. The design considerations for a microchannel network include the length, the hydraulic diameter, the channel shape, the channel junctions, thermal management, and flow control. For laminar, single-phase flow of a Newtonian fluid, the researcher or developer may apply the conventional laminar flow theory for network design with consideration to the above mentioned design factors. However, the characterization of bio-flow is generally not modeled easily using conventional laminar theory since biological fluids tend to be non-Newtonian and multiphase. The characterization of bio-flow and the performance of bio-devices, in many instances, must be experimentally studied in order to fulfill the specific purpose of design.

The IBC concept is to separate the microchannel network, typically in the form of a thin chip type plate, from the external sources for flow potential and the diagnostic or sensing devices. Ideally, micro-bio-systems would incorporate microscale pumps and sensors within the chip, but micropumps and microsensors are insufficiently developed at this time.

Given the limitation of suitably validated pumping and sensing microdevices, the most common use of microchannels in biological research is for mixing, separating, and reacting. Typically, the flow is driven via external pressure imbalances along the channel. Thus, the IBC concept is ideally suited for repetitive experiments involving micro-bio-flow experiments because the operating conditions can easily be manipulated.

Potential for Microdevice Development

The most significant application of the IBC system may be as a platform for testing the behavior of a microfluidic subsystem that will eventually be integrated into a complete microelectromechanical systems (MEMS) device. There are multitudes of microfluidic-based devices currently under development. A small sampling includes systems for micro-dosing and drug delivery, monitoring of medical conditions, sampling of airborne particles or contaminants, automated drug performance testing and genetic testing [Koch et.al., 2000]. Each of these devices is comprised of a number of subsystems and each subsystem needs to be suitably modeled or validated.

The IBC platform may be used to validate subsystem performance for microdevices. Devices such as flow sensors, chemical sensors, thermal sensors, etc. may be calibrated. The efficacy of diffusive and chaotic mixing may be tested under widely varying flow conditions. The performance limits of a micropump may be determined for a range of pressure heads. In addition, submodels may be validated for use in a computational simulation of the entire microdevice.

FUTURE DEVELOPMENT

In the near future, IBC will strive to expand the use of microfluidics to other science disciplines within the NASA agency.

A significant feature of the unit being developed is the web-based interface to the Internet. Similar to how IBC developed capabilities for telescience, an investigator may have access to and limited control of the operations via the Internet. In this manner, units may be accessed by researchers who have limited budgets and or needs. Finally, with microfluidics being a new and expanding field, the unit may be implemented for educational purposes too.

For flight related activities, many capabilities need to materialize for the chip to be truly optimized in its usage. One needed feature is a true controllable micro-isolation valve on the chip. This will allow for the preloading and storing of unmixed chemicals on the chip. It is also needed for isolating reactor wells prior to operation. The use of valving will also allow for even greater control of fluidic movement on a chip. One key factor is that the valve must be a normally closed valve, meaning no power or other external influence is needed to maintain valve closure.

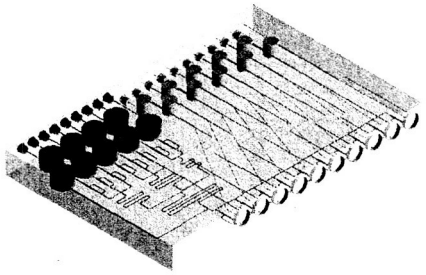


Fig. 7 Schematic concept of a flight chip with integrated microsystems and embedded waveguides.

Another issue to be resolved is the sealing of a chip to prevent internal loss of fluids and external contamination. These seals will need to accept the interfaces of the macro world for chip loading, analysis, and fluidic processing. This will be followed by incorporation of sensors onto the chip. A concept chip is shown in Figure 7. These sensors will be used to measure local fluidic temperatures, pressures, flow rates, pH, etc. The sensors will aid in not only the handling of microfluidics but also provide expansion to analytical capabilities. The final near term feature required will be the incorporation of microfluidic pumps into the chip. This would significantly reduce weight, volume, power consumption, macro world interface complexity, and increase overall systems reliability. All of these issues are areas of concern for space flight.

Having demonstrated the implementation of some of these features individually on a chip, we will then move on to demonstrating them as part of a complete system. With a tool such as the ground development unit planned, these goals will be met.

CONCLUSION

With these new tools and operating systems, it will make possible the emergence of new technical disciplines such as micro-hydraulics. With time, micro-hydraulics (microfluidics) will be used in medicine, our cars, the military, toys, home appliances, and a multitude of other areas. The IBC team is greatly interested in the variety of diagnostics and features that other communities would like to see incorporated into its ground development unit.

NOMENCLATURE

cp Centipoise
μA Microamps
μL Microliters
μm Microns
mm Millimeters
nA Nanoamps
psi Pounds per square inch
V Voltage
X Magnification

ACKNOWLEDGEMENTS

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